Video Article

Creation of Abdominal Adhesions in Mice

Clement D. Marshall[1], Michael S. Hu[1], Tripp Leavitt[1], Leandra A. Barnes[1], Alexander T.M. Cheung[1], Samir Malhotra[1], H. Peter Lorenz[1], Michael T. Longaker[1]

[1]Hagey Laboratory for Pediatric Regenerative Medicine, Division of Plastic and Reconstructive Surgery, Department of Surgery, Stanford University School of Medicine

Correspondence to: Clement D. Marshall at cmarshal@stanford.edu

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Abstract

Abdominal adhesions consist of fibrotic tissue that forms in the peritoneal space in response to an inflammatory insult, typically surgery or intraabdominal infection. The precise mechanisms underlying adhesion formation are poorly understood. Many compounds and physical barriers have been tested for their ability to prevent adhesions after surgery with varying levels of success. The mouse and rat are important models for the study of abdominal adhesions. Several different techniques for the creation of adhesions in the mouse and rat exist in the literature. Here we describe a protocol utilizing abrasion of the cecum with sandpaper and sutures placed in the right abdominal sidewall. The mouse is anesthetized and the abdomen is prepped. A midline laparotomy is created and the cecum is identified. Sandpaper is used to gently abrade the surface of the cecum. Next, several figure-of-eight sutures are placed into the peritoneum of the right abdominal sidewall. The abdominal cavity is irrigated, a small amount of starch is applied, and the incision is closed. We have found that this technique produces the most consistent adhesions with the lowest mortality rate.

Video Link

The video component of this article can be found at http://www.jove.com/video/54450/

Introduction

Abdominal adhesions are a form of scar tissue that form in the abdomen in response to inflammation, typically following surgery or intraabdominal infection. Adhesions are a major cause of chronic abdominal pain and infertility, and are the most common cause of small bowel obstruction[1]. The presence of adhesions makes performing a second abdominal operation more difficult and increases the likelihood of complications[2].

Despite years of research, the mechanisms underlying the formation of adhesions remain poorly understood. It is known that an initial injury to the peritoneal surface causes an exudation of fibrin-rich fluid, which then forms a clot that binds the surfaces of bowel and the abdominal wall together[3]. Later, fibroblasts and other cells migrate into the adhesive space and secrete connective tissue[4]. Over months to years the adhesion matures by developing blood vessels and nerves[5].

Several commercial products exist that are designed to reduce the formation of adhesions after abdominal surgery (*e.g.*, Seprafilm). All of these products act as mechanical barriers and stop adhesion formation by preventing physical contact between loops of bowel and the abdominal wall[6,7]. Despite evidence from a controlled trial that a surgical adhesion barrier reduces the formation of adhesions[8], many surgeons anecdotally have been disappointed with the effectiveness of mechanical barrier products.

Currently there are no drug-based anti-adhesion therapies, which reflects the fact that the precise processes involved in adhesion formation are poorly understood. Developing a therapy that specifically targets cellular or molecular agents involved in the formation of adhesions will require an improved understanding of the events that are involved in the formation of the adhesion. Several groups have identified molecular pathways that may be important for adhesion formation[9-11]. Animal models provide a superb environment for studying the formation of adhesions. Many studies have been published describing the surgical creation of adhesion in several animals, particular the rat and mouse[6,12-14]. Given our experience with studying fibrosis in the mouse and the wide availability of transgenic mice and mouse-based antibodies, we chose the mouse as our model for the study of adhesions. Herein, we report the technique that we have developed to reproducibly and reliably create abdominal adhesions in the mouse.

Protocol

The following protocol has been approved by the Stanford University Institutional Animal Care and Use Committee (IACUC) and complies with all institutional ethical guidelines regarding the use of research animals.

1. Creation of Abdominal Adhesions

1. Start the mouse on antibiotic chow diet one week prior to the procedure.
2. Autoclave the surgical instruments and pre-warm the saline irrigation solution.
3. Anesthetize the mouse using 2% inhaled isoflurane.
4. Using a hair clipper followed by a quick application (5 - 10 sec) of depilatory agent, remove the hair from the majority of the surface of the abdomen. Thoroughly remove the depilatory agent by gently wiping with wet gauze and, optionally, by carefully dunking the lower half of the mouse into warm water. Thoroughly dry the animal.
5. Place the animal's snout into the anesthetic nose cone. During the entire surgery, carefully monitor the respiratory rate of the animal and titrate the anesthetic flow rate as needed.
6. Use a warming device, such as a heating pad or a warming lamp, to prevent hypothermia.
7. Prior to prepping the abdomen, secure the mouse with strips of tape placed above and below the abdomen to prevent the mouse from shifting during surgery. Disinfect the abdomen with betadine, then follow with 70% ethanol. Include as much area as possible in the prep, including the hair on the edges of the shaved area. Place a sterile drape over the mouse. (**Figure 1**)
8. Subcutaneously inject 0.1 mg/kg buprenorphine, or 3 µg for a 30 g mouse.
9. Make the skin incision.
    1. Starting in the lower abdominal midline, grasp the skin with forceps and make a shallow vertical cut in the skin using sharp scissors.
    2. Carry the cut superiorly to the xyphoid with the scissors. Take care to enter only the skin.
10. As the abdominal musculature is now visible covering the viscera, grasp the midline of the musculature with forceps and very carefully make a small cut into it using sharp scissors. Make sure not to accidentally cut into an abdominal organ.
11. After entering the peritoneal space with a small cut and seeing the muscular layer pull away from the abdominal organs, extend the cut superiorly and inferiorly by inserting the scissors into the opening and carefully cutting in both directions. Extend the incision from the xyphoid superiorly to above the bladder inferiorly. (**Figure 2**)
12. Now that the intestines are exposed, locate the cecum and gently exteriorize it. Avoid grasping the cecum with toothed or sharp forceps. Instead use an atraumatic forceps such as an Adson serrated forceps.
13. Abrade the cecum with sandpaper.
    1. Gently abrade the entire surface of both sides of the cecum with 100 grit sandpaper for 30 - 60 sec until the surface becomes less shiny and petechiae appear on the surface. If the surgeon is right-handed, it helps to orient the cecum with the tip facing to the right and drape the cecum over the surgeon's left index finger. (**Figure 3**)
    2. Perform the sanding gently, as it is easy to accidentally cause a perforation of the thin cecal wall. A small amount of grittiness should be felt as the sandpaper is moved along the cecal surface. If the sandpaper is felt repeatedly catching on the cecum, this will likely cause a tear in the cecal wall. Watch carefully to ensure that particles of sandpaper do not dislodge and remain in the abdomen, as this may cause excessive foreign body reaction.
    3. If a tear in the cecal wall occurs, terminate the procedure and euthanize the animal.
    4. If a cecal blood vessel is sheared by the sandpaper and causes bleeding, hold pressure for up to two minutes with gauze. However, if bleeding persists after this time, place a figure-of-eight 7-0 monofilament suture around the point of bleeding. This will reliably stop the bleeding in almost all cases. Take care not to incorporate a large amount of cecal wall into the suture, as this risks causing necrosis of the involved part of the wall.
14. Injure the right abdominal sidewall.
    1. Use a strip of sandpaper to abrade the peritoneal surface of the muscle of the right abdominal sidewall. Be more aggressive with the sanding here than on the cecum and continue until the entire surface of the right abdominal sidewall appears roughed up. Avoid sanding so hard that skin is visible through openings in the muscular layer. (**Figure 4**)
    2. Using a Castro-Viejo needle driver, place between two and four figure-of-eight 4-0 silk stitches into the muscular layer of the right abdominal sidewall. Leave the tails about 5 mm long. Take care not to accidentally catch bowel wall into the suture. (**Figure 5**)
15. Using a 10 ml syringe filled with warmed saline, irrigate the intestines several times. Direct the stream of saline into the abdominal cavity to irrigate the interior as well. If the surface underneath the mouse becomes soaked, move the mouse over or replace the surface, in order to avoid hypothermia.
16. Use a sterile gauze placed over the incision to soak up excess irrigation.
17. Take a pinch of starch and sprinkle it onto the surface of the right abdominal sidewall and onto both sides of the cecum. (**Figure 6**)
18. Make sure that there is no active bleeding. If there is, use a gauze sponge to apply pressure directly onto the point of bleeding until it stops. If the bleeding does not stop readily, place a hemostatic suture as described above.
19. Using the blunt end of the forceps and a finger, gently push the intestines back into the abdominal cavity. Position the cecum next to the sutures in the right abdominal sidewall in order to maximize adhesion formation.
20. Close the abdominal incision.
    1. Using 6-0 absorbable braided suture, place a running stitch into the muscular layer at the top of the incision.
    2. Run the suture down toward the bottom of the incision, bringing together the muscular layer. Travel by about 3 mm and take 3 mm bites with each new stitch. Take care not to accidentally take a bite of intestine while placing sutures.
    3. At the bottom of the incision, leave a loop of suture from the previous bite and use this to instrument tie the suture. Cut the suture leaving 5 mm tails.
    4. Repeat the above three steps with 6-0 nylon monofilament suture to close the skin.
21. Administer a 20 ml/kg subcutaneous saline bolus (roughly 0.5 ml for a 30 g mouse).
22. Using a dry gauze, thoroughly dry the entire animal, as the dorsal fur tends to get wet during irrigation.
23. Loosely wrap an adhesive dressing around the abdomen to cover the incision. Take care not to constrain the animal's legs or breathing mechanics with the dressing.

24. Carefully monitor the animal as it recovers from anesthesia. Administer buprenorphine every 12 hr for 2-3 days after the procedure for pain control.

2. Harvesting Adhesion Tissue

1. Wait a minimum of seven days after the initial surgery. Again, autoclave surgical instruments and anesthetize the mouse with isoflurane.
2. Sterilize the abdomen with betadine followed by 70% ethanol. Shave and depilate additional hair from the right side of the abdomen so that the adhesion specimen containing skin will not be covered in hair, which makes histology more difficult.
3. Use a left paramedian incision, since bowel is often stuck to the original incision. Starting at the lower end of the abdomen, about 5 mm to the left of the bottom of the original incision, grasp the skin with small toothed forceps and cut into the skin with sharp scissors. Extend this cut superiorly to the ribcage. (**Figure 7**)
4. Using scissors, cut into the muscular layer and after entering the peritoneal space, extend the cut to the top and bottom of the incision. There will be adhesive tissue causing the cecum and small bowel to adhere to the right sidewall of the abdomen and possibly also to the original incision. (**Figure 8**)
5. Using the scissors and starting on the edge of the incision, above the level of the cecum, start cutting into the abdominal wall in a circle around the area where the bowel is adherent.
6. Alternating above and below the level of the cecum, gradually cut all the way around the adhesion and complete the circle, yielding an "island" of skin and abdominal wall adherent to the bowel. (**Figure 9**)
7. Again using the scissors, cut into the bowel and separate the adherent part from the rest of the bowel, yielding a sandwich of tissue that consists of skin and abdominal wall on one side, bowel on the other side, and adhesive tissue connecting the two in between. (**Figure 10**)
8. Optionally, use a pointed scissors to sharply separate the skin from the abdominal wall muscle. For histology consider leaving the skin attached, but for digestion and cell isolation the skin can be removed. If the tissue will be used for histology, make sure to remove as much suture material as possible without excessively disturbing the adhesive interface. For digestion and cell isolation it is acceptable to leave the sutures in place as long as the tissue digest is filtered.
9. After excising adhesion tissue, euthanize the mouse using a humane method approved by your institutional animal use committee.

Representative Results

At seven days after surgery, the cecum and possibly ascending colon, liver, and loops of small bowel should be adherent to the right-sided abdominal wall. (**Figure 8**) Excised tissue can be embedded and sectioned and will yield excellent histological slides. (**Figure 11, 12**)

When the procedure is performed properly, 100% of mice should have substantial adhesions at seven days. Mortality should be less than 5%.

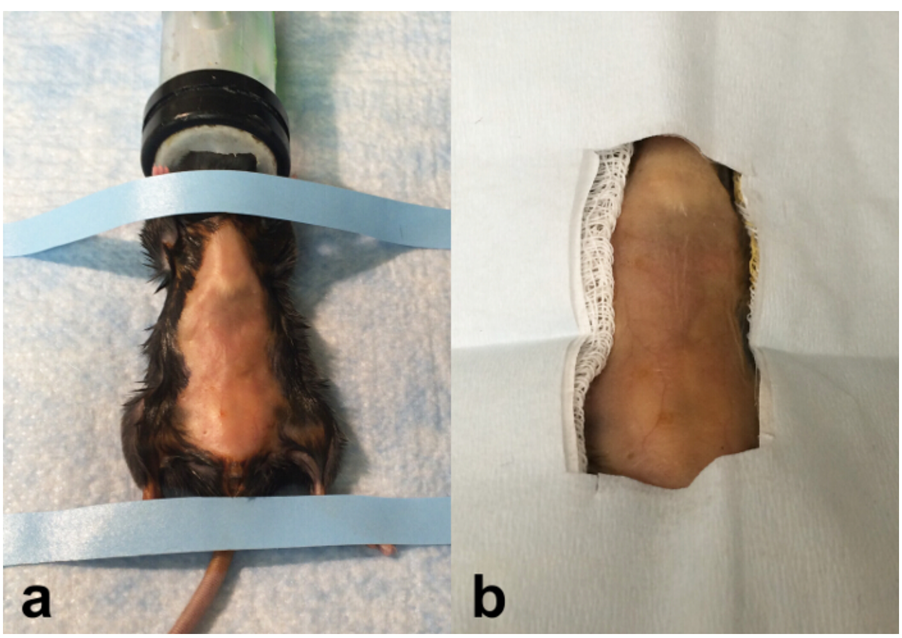

Figure 1: Preparation for Surgery. (a) The animal has been secured with tape and the abdomen prepped with betadine. **(b)** The betadine has been cleared with an alcohol swab and a sterile drape has been placed. Please click here to view a larger version of this figure.

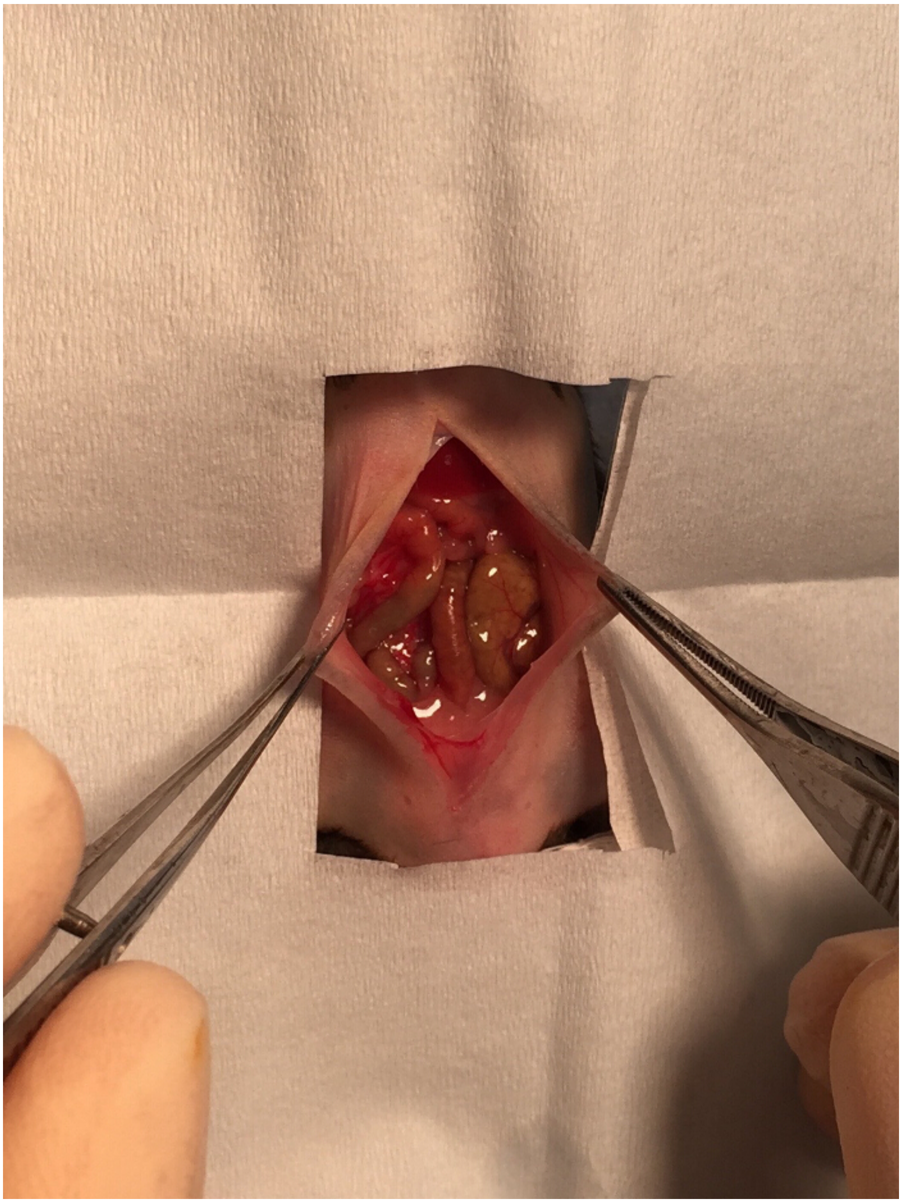

Figure 2: Incision. The incision should extend from the level of the bladder to the xyphoid. Please click here to view a larger version of this figure.

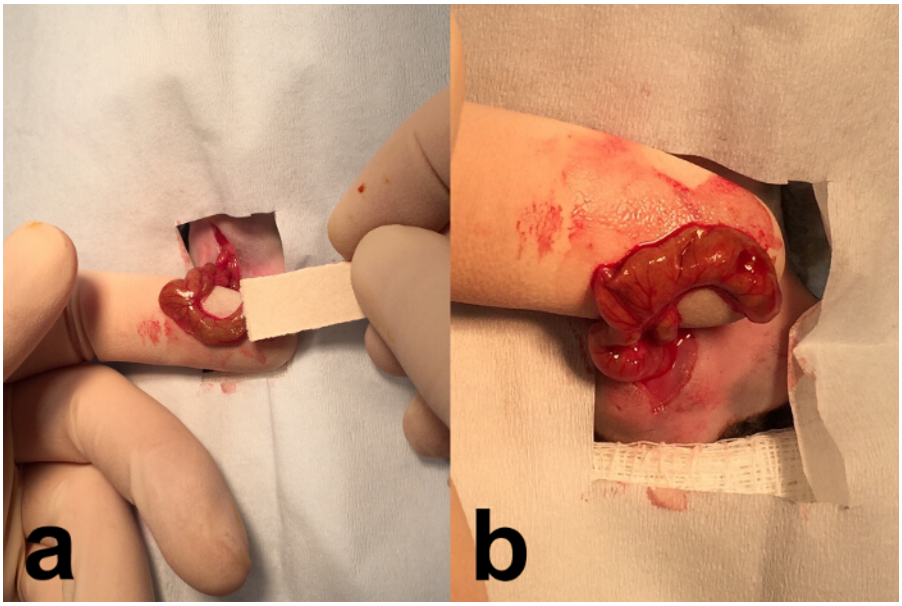

Figure 3: Abrasion of the Cecum. (a) Draping the cecum over the left index finger with the tip pointing to the right allows the base of the cecum to be stabilized by the left thumb. **(b)** An appropriate level of sanding leaves the cecum with a rough surface that is less shiny than before, and with several petechial points of bleeding. Extra care should be taken while sanding near the mesenteric side, as the vessels here will bleed the most briskly. Please click here to view a larger version of this figure.

Figure 4: Abrasion of the Abdominal Wall. The viscera are swept aside and the peritoneal surface of the muscle wall is sanded until it appears rough. The blood vessel seen just above the sandpaper in the abdominal wall is frequently encountered but will not bleed substantially. Please click here to view a larger version of this figure.

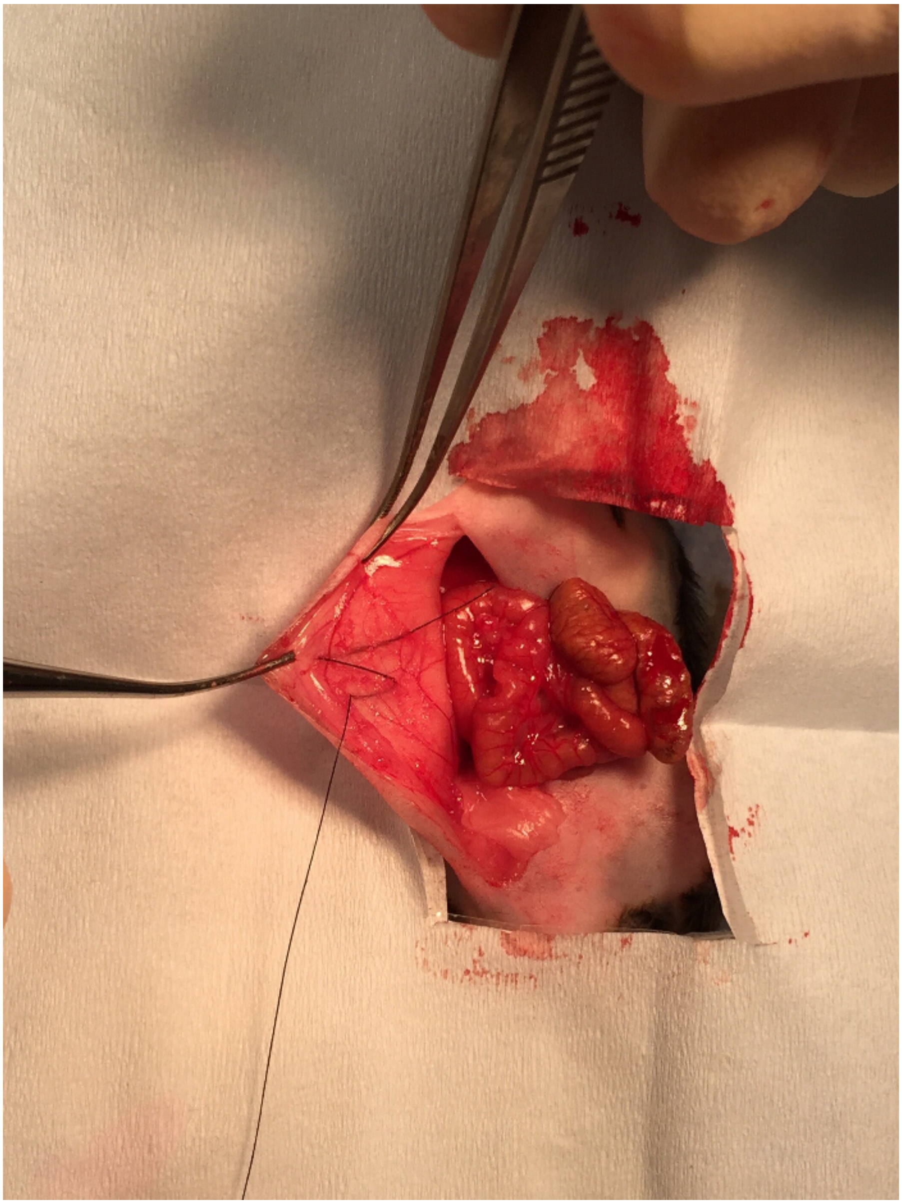

Figure 5: Placement of the Figure-of-eight Suture. A silk figure-of-eight suture placed into the muscle wall, prior to being tied down. Please click here to view a larger version of this figure.

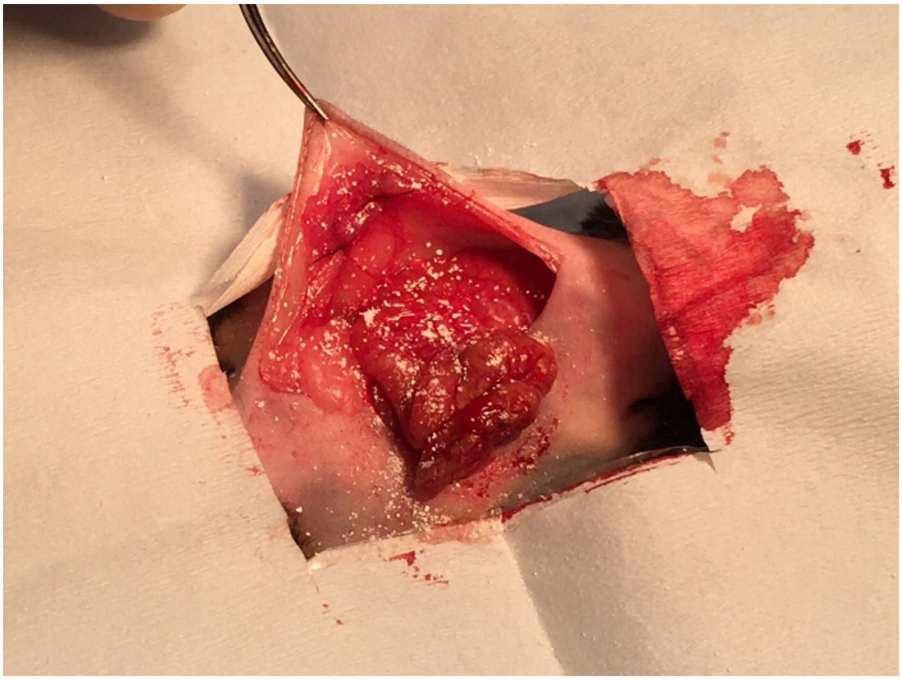

Figure 6: Application of Starch. A light layer of starch is sprinkled over the abdominal sidewall and bowel. Two figure-of-eight sutures are seen in the muscle wall. Please click here to view a larger version of this figure.

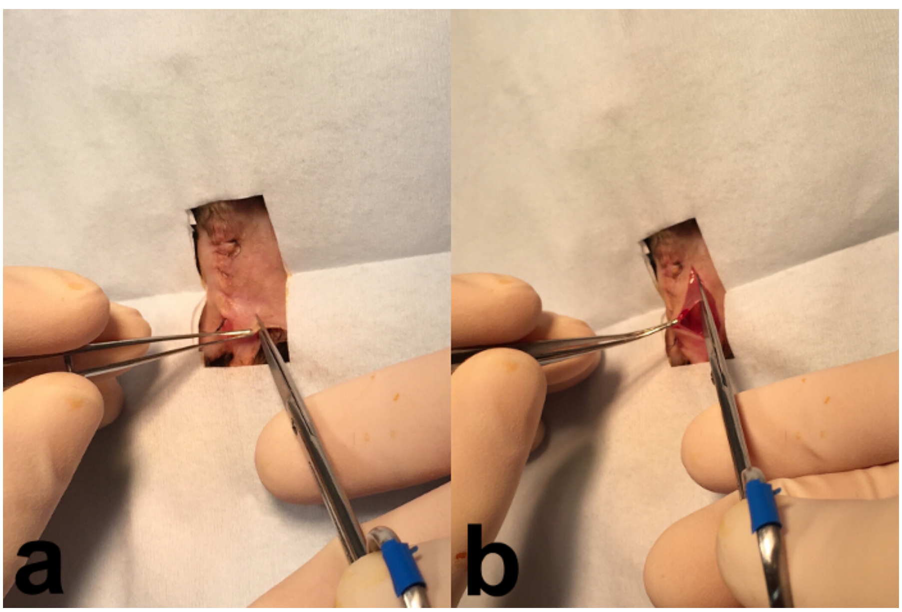

Figure 7: Incision for the Second Surgery. (a) The incision is started just to the left of the original incision. **(b)** The new incision should extend from the level of the bladder to the ribcage. Please click here to view a larger version of this figure.

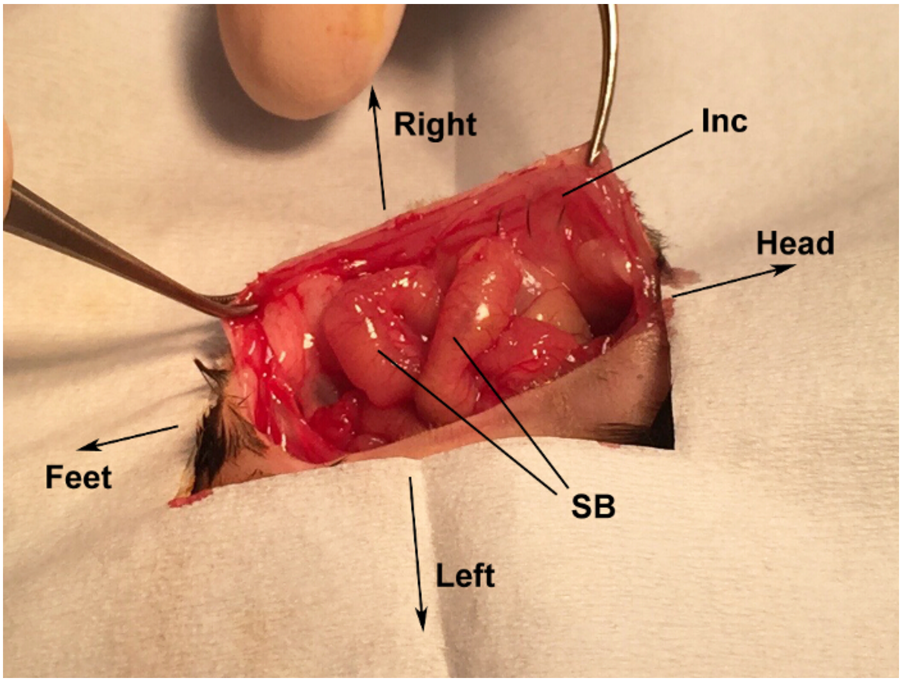

Figure 8: The Adhesion. Loops of small bowel (SB) are adherent to the right sidewall and to each other. The original midline incision, now healed, is seen (Inc). Please click here to view a larger version of this figure.

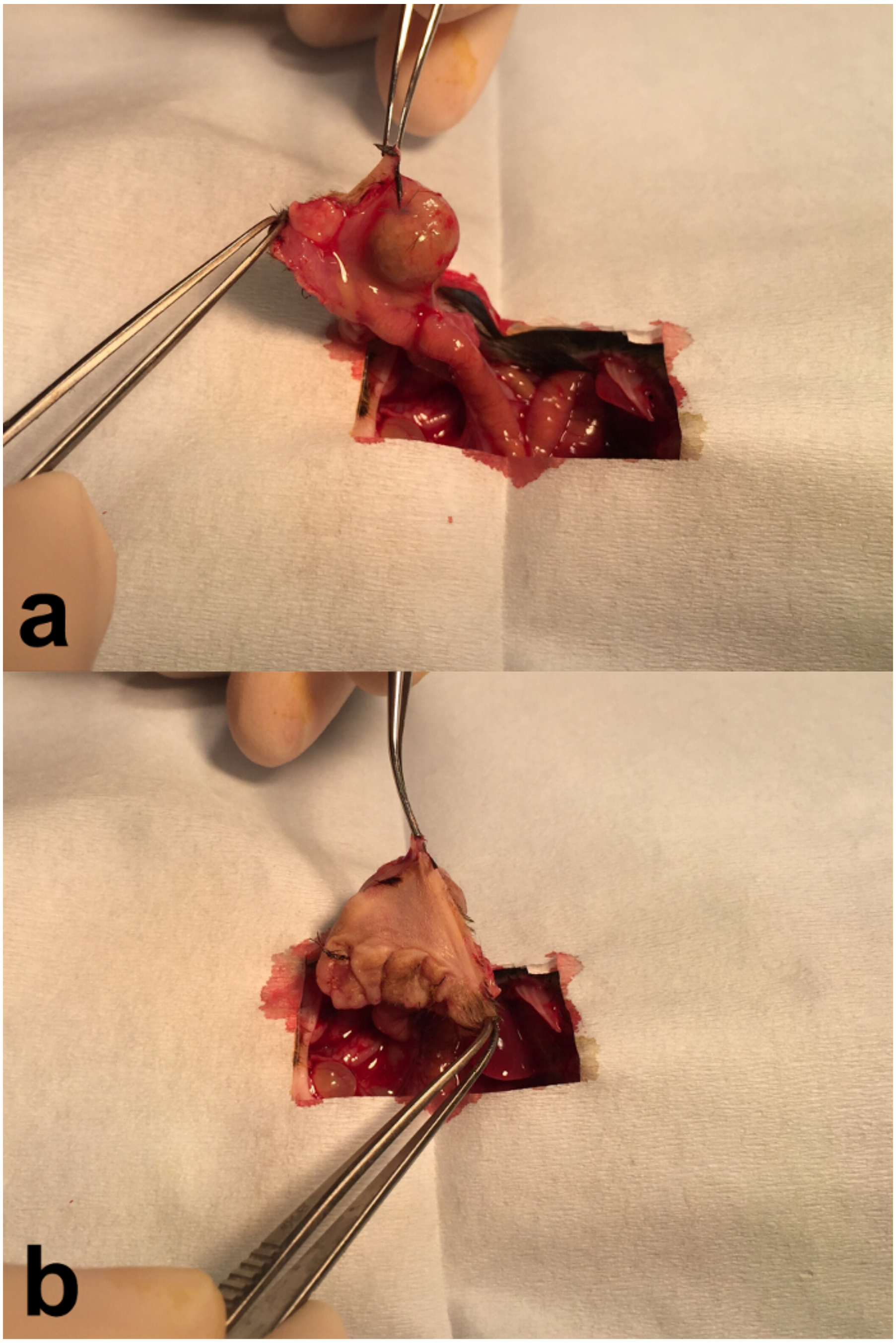

Figure 9: Excising Adhesion Tissue. (a) and **(b)** An "island" of skin and abdominal wall adherent to the underlying bowel is freed by cutting in a complete circle around the adherent area. Please click here to view a larger version of this figure.

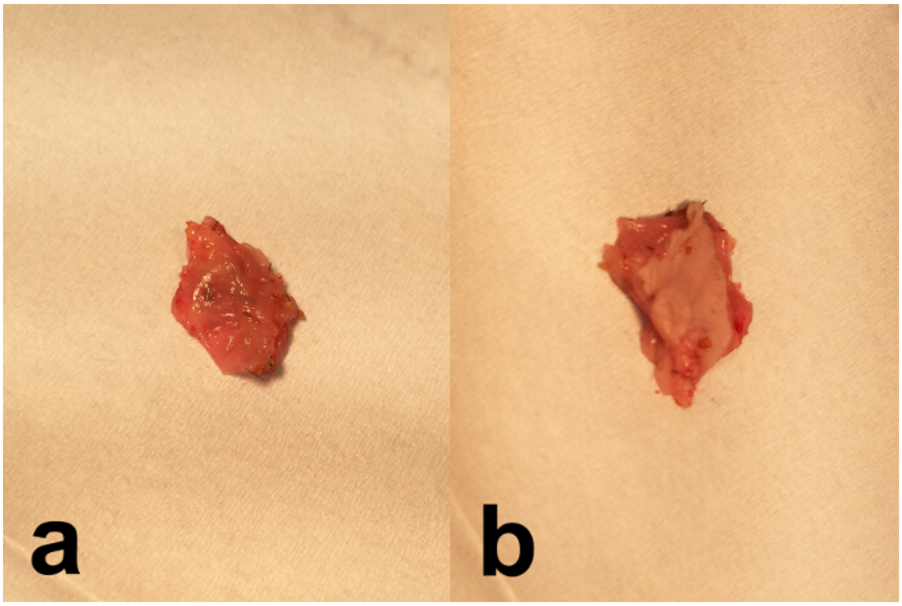

Figure 10: The Specimen. Cutting across the bowel will yield a sandwich of tissue with bowel on one side **(a)** and skin on the other side **(b)**. The pieces of suture visible should be removed if the specimen is to be used for histology. Please click here to view a larger version of this figure.

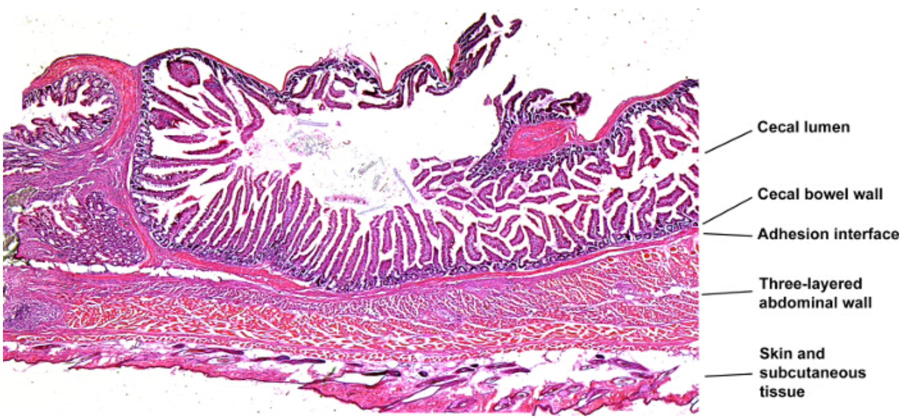

Figure 11: Histology. A hematoxylin and eosin (H&E) stained section of an adhesion at seven days after surgery showing skin and abdominal wall (bottom) attached via the adhesive interface to the cecum (top). At this early time point the cecum is likely adhered to the abdominal wall mainly through fibrin and other molecules, and substantial scar tissue has not yet formed. Please click here to view a larger version of this figure.

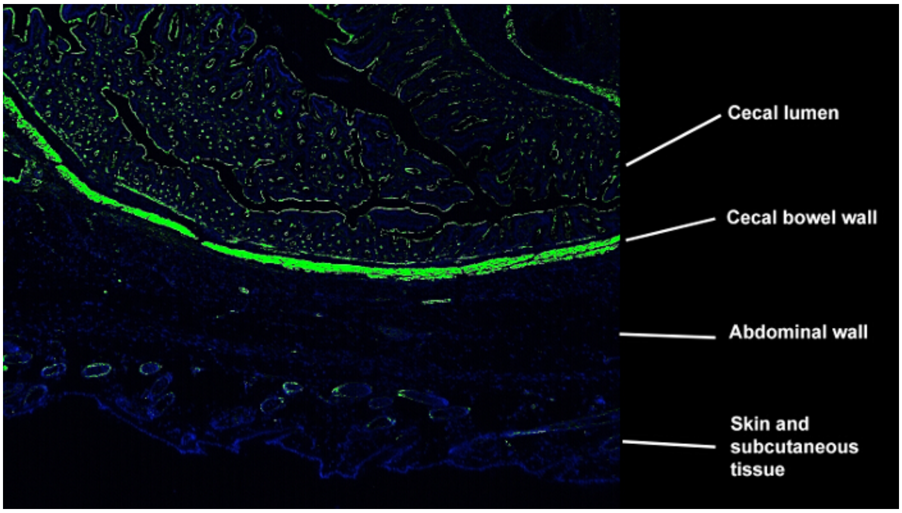

Figure 12: Immunofluorescent Imaging. A section of adhesion at seven days after surgery immunofluorescently stained for α-smooth muscle actin, showing true staining of the skin hair follicles (left) and cecal muscle wall (middle), and non-specific staining of the cecal luminal surface (right). Please click here to view a larger version of this figure.

Discussion

The critical steps in this procedure are: thoroughly abrading the cecum without causing perforation, placing sutures in the abdominal sidewall, and applying the right amount of starch. Only apply sandpaper to the cecum, or to a small specific portion of the bowel. Wide use of sandpaper on large amounts of small bowel tends to cause significant ileus. Take care to abrade the cecum with enough force that the surface becomes rough, but not so much that the wall tears. Finding this balance can take some time. Always handle the intestine carefully and avoid using sharp or toothed forceps to manipulate bowel. It is easy to accidentally cause bleeding by grabbing the small bowel mesentery with forceps or by pulling on the bowel too forcefully.

If a tear occurs in the cecal wall, the procedure should be terminated and the animal euthanized. In our experience, cecal tear results in death of the animal after a few days, even if the tear is repaired well. In addition, tear of the cecal wall will cause spillage of stool that will affect the inflammatory response in an unpredictable way.

Hemostasis must be complete at the end of the procedure. Some bleeding vessels will re-open and start to bleed after appearing to have stopped. When in doubt, place an absorbable monofilament figure-of-eight suture around the point of bleeding. This type of suture is preferable to silk or braided absorbable suture because it can be pulled though the tissue with minimal resistance. However we have found that silk or braided absorbable suture is most effective in the abdominal sidewall for inducing adhesions, as monofilament is less inflammatory.

Application of starch at the end of the procedure helps to increase the formation of adhesions, but we have discovered that an excessive amount of starch will cause an inflammatory reaction that causes death. The starch should be sprinkled on lightly. There should not be so much that it forms a solid layer.

When first learning this procedure, it is common to lose several mice. In our experience the most common causes of death are dehydration due to ileus caused by overly vigorous abrasion of intestine, and sepsis due to intestinal perforation. Death caused by bleeding can occur if complete hemostasis is not achieved at the end of the surgery.

If adhesion formation is inadequate, consider using more aggressive abrasion of the cecum and right sidewall, leaving more starch, or placing more sutures on the abdominal sidewall. In our experience, waiting for less than a week before reopening the abdomen results in less than adequate adhesion formation. On the other hand, if the mortality rate of mice after surgery is high, consider using less aggressive abrasion of the cecum, inspecting more closely for hemostasis, and applying less starch. It is also important to carefully monitor the respiratory rate of the mouse during surgery. Once the surgeon gains experience with this procedure, mortality should be less than 10%.

This technique is designed to primarily form adhesions between the cecum and the right abdominal sidewall. Adhesions will also often form between small bowel, liver, and the midline incision. Using this technique will usually not cause adhesions on the left side of the abdomen. A limitation of this technique is that adhesions between bowel and abdominal wall are more likely to form than adhesions between loops of bowel. In addition, it is not likely to result in a totally frozen abdomen full of adhesive tissue, which is often seen in humans who have had multiple surgeries.

As discussed in the introduction, many techniques for producing abdominal adhesions in mice and other species, particularly rat, have been described in the literature[6,12-14]. There are far fewer published protocols for the creation of adhesions in mice. We speculate that because the creation of adhesions in mice is more difficult than in rats, most investigators chose to develop their technique in the rat. However, because of the greater availability of transgenic mice and anti-mouse antibodies, we believe that it is valuable to have a robust model for adhesion creation in mice, despite the greater technical difficulty. We developed this protocol after trying many of the published rat protocols. We found that methods for creating adhesions in the rat, such as the use of electrocautery on the cecum, often cause mice to die. We were not satisfied with the density of adhesions produced by techniques that use a single intervention, such as placing sidewall ischemic buttons alone, or only abrading the cecum. The technique we present here represents a combination of interventions that we found, through trial and error, to produce adhesions consistently while minimizing mortality.

Because this technique consistently causes adhesive tissue to form between the cecum and the sidewall, we believe that it is ideal to test interventions designed to reduce adhesion formation. Also this technique can be used to explore the molecular pathways and cell types that are involved in adhesion formation. The adhesion tissue can easily be excised and prepared for histology, and yields excellent histological images (**Figures 11, 12)**.

Some researchers may desire a model in which adhesions form less than 100% of the time. Omitting the application of starch will reduce the adhesion rate to roughly 80%. Decreasing the number of silk sutures placed into the right abdominal sidewall will reduce the adhesion rate further.

In our experience, day seven after surgery is the first time point where the bowel is consistently adherent to the abdominal wall. However, other time points may be more relevant depending on the focus of the project. For example, researchers interested in the migration of neutrophils and macrophages into the adhesion space may want to harvest tissue in the first five days after surgery. On the other hand, the deposition of collagen by fibroblasts takes place for weeks after the initial injury, and for this it will be more appropriate to examine tissue at time points spaced out for several weeks after surgery.

Surgical loupes are very helpful for magnification of the surgical field while performing this procedure. An operative microscope can also be used but limits the surgeon's ability to inspect the operative field from different angles. The procedure can also be done with no magnification at all.

Disclosures

The authors declare that they have no competing financial interests.

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